

WE CLAIM:

1. A method of inducing at least one site-directed double-strand break in DNA of a cell, said method comprising
  - (a) providing cells containing double-stranded DNA, wherein said DNA comprises at least one I-Sce I restriction site;
  - (b) transfecting said cells with at least a plasmid comprising DNA encoding the I-Sce I meganuclease; and
  - (c) selecting cells in which at least one double-strand break has been induced.
2. The method of claim 1, wherein said cell is selected from the group consisting of a mammalian cell, a yeast cell, and a plant cell.
3. The method of claim 2, wherein said cell is an NIH3T3 cell containing the G-MtkPL virus.
4. The method of claim 1, wherein said plasmid is pCMV(I-Sce I+).
5. A method of inducing homologous recombination between chromosomal DNA of a cell and exogenous DNA added to said cell, said method comprising
  - (a) providing cells containing chromosomal DNA, wherein said DNA comprises at least one I-Sce I restriction site;
  - (b) transfecting said cells with a plasmid comprising exogenous DNA, and with a plasmid comprising DNA encoding the I-Sce I meganuclease; and
  - (c) selecting cells in which said exogenous DNA is inserted into said chromosomal DNA.

00492697-012700

6. The method of claim 5, wherein said cell is selected from the group consisting of a mammalian cell, a yeast cell, and a plant cell.

7. The method of claim 6, said cell is an NIH3T3 cell containing the G-MtkPL virus.

8. The method of claim 5, wherein said plasmid is pCMV(I-Sce I+).

9. A method of inducing homologous recombination between chromosomal DNA of a cell and exogenous DNA added to said cell, said method comprising

(a) providing cells comprising chromosomal DNA;  
(b) inserting at least one I-Sce I restriction site in said chromosomal DNA;

(c) transfecting said cells with a first plasmid comprising exogenous DNA, and with a second plasmid comprising DNA encoding the I-Sce I meganuclease; and

(d) selecting cells in which said exogenous DNA is inserted into said chromosomal DNA.

10. The method of claim 9, wherein said cell is selected from the group consisting of a mammalian cell, a yeast cell, and a plant cell.

11. The method of claim 9, wherein said first plasmid is pCMV(I-Sce I+).

12. The method of claim 9, wherein said second plasmid is pVRneo.

13. A method of inducing at least one site-directed break in DNA of a cell and inserting DNA encoding a polypeptide, said method comprising,

00492697.012700

(a) providing cells containing double-stranded DNA, wherein said cells are capable of being transformed by a DNA comprising a I-Sce I restriction site and DNA encoding said polypeptide;

(b) adding *Sce* I enzyme or transforming said cell with DNA encoding *Sce* I enzyme;

(c) transfecting said cells with said DNA encoding said polypeptide or with a vector containing said DNA; and

(d) selecting cells transfected with said DNA or said vector, wherein said cells express said polypeptide.

14. A recombinant eukaryotic cell transformed by the method of any one of claims 1 and 13.

15. A transgenic animal comprising a cell transformed by the method of any one of claims 1 and 13.

16. A method of expressing a polypeptide in a transgenic animal, said method comprising transforming embryonic stem cells with a DNA comprising a I-Sce I restriction site and DNA encoding said polypeptide, and detecting expression of said polypeptide in a transgenic animal resulting from said transformed embryonic stem cells.

17. A recombinant stem cell expressing a polypeptide, wherein said stem cell is transformed by a DNA comprising a I-Sce I restriction site and DNA encoding said polypeptide by

(a) adding *Sce* I enzyme to said cell or transforming said cell with a vector containing the gene coding for *Sce* I enzyme;

(b) transfecting said cells with said DNA encoding said polypeptide; and

(c) selecting cells transfected with said DNA, wherein said cells express said polypeptide.

004270 26926460

18. A recombinant eukaryotic cell as claimed in any one of claims 4 and 7 wherein said polypeptide is a foreign antigen to the cell.

19. The recombinant eukaryotic cell as claimed in claim 14 wherein cell is a mammalian cell line.

20. The recombinant eukaryotic cell as claimed in claim 14 wherein cell is a yeast.

21. A method of inducing at least one site-directed break in DNA of cells and inserting DNA encoding a polypeptide, wherein said cells express at least one protein product, said method comprising,

(a) providing cells containing double-stranded DNA, wherein said cells are capable of being transformed by a DNA comprising a I-Sce I restriction site and DNA encoding said polypeptide;

(b) adding Sce I enzyme to said cells or transforming said cells with DNA encoding Sce I enzyme;

(c) transfecting said cells with said DNA encoding said polypeptide or with a vector containing said DNA; and

(d) selecting cells transfected with said DNA or said vector, wherein said cells express said polypeptide and do not express said protein product.

22. A recombinant cell transformed by the method of claim 21.

ADD A3  
ADD E'  
add F1

00492697-012100